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(54) Apparatus and method for detecting a substance

(57) An apparatus for detecting a substance in a sample, particularly for in vivo detecting and measuring glucose in body tissue or blood contains a semiconductor laser (4,5) for emitting mid-infrared laser light (10) at at least two discrete wavelengths, each at a different peak or valley in the absorption spectrum of the substance in the sample. A photoacoustic detector (3,6) detects acoustic signals (11) originating from absorption of the laser light. An indication unit (7) evaluates the acoustic signals separately for each wavelength and calculates a detection result based on all acoustic signals from the different wavelengths.

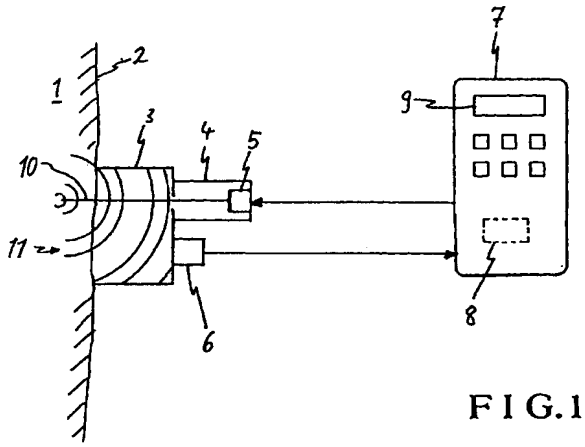

FIG.1

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Description

[0001] The invention relates to an apparatus and a method for detecting a substance in a sample, particularly for detecting and measuring the concentration of a substance such as glucose in body fluid or tissue.

[0002] Insulin dependent diabetics have to monitor their blood glucose concentrations at regular intervals. At present, this is mostly done by taking a blood sample and analysing it outside the patient's body. Patients who monitor their blood glucose level themselves use a finger lance to obtain a drop of blood which is applied to a reagent strip for analysing. Naturally this process causes pain and discomfort. There have been various attempts, therefore, to detect blood glucose concentrations in vivo.

[0003] EP-A-282234 proposes in vivo detection of glucose in the blood stream by infrared spectroscopy using a laser beam penetrating a person's skin. The wavelength of the laser beam is selected in the near-infrared (NIR) range of 0.76 to 2.5 μm.

[0004] As explained by H.A. Mac Kenzie et al. in Phys. Med. Biol. 38 (1993) 1911-1922 and in Clinical Chemistry 45:9 (1999) 1587-1595, the near-infrared range and in particular the wavelength region of 1 to 2 μm is preferred for non-invasive blood glucose measurement as the absorption of light of other wavelengths in the human skin is too large for the light to penetrate to a suitable depth for interaction with blood. In Medical & Biological Engineering & Computing, May 1993, 284 to 290, Mac Kenzie et al. report also the use of the mid-infrared (MIR) wavelength region of 2.5 to 25 μm for glucose measurements. But due to the very low skin transmission at these wavelengths, they have not measured glucose concentrations in vivo. The mid-infrared light source used is a CO_2 laser and the glucose concentration is obtained by measuring the absorption coefficient in the sample at a certain wavelength and relating it to the absorption coefficient of distilled water at the same wavelength.

[0005] In the above-discussed prior art, the optical absorption coefficient is measured through the photoacoustic effect: optical absorption of infrared radiation leads to molecular resonance such as vibrational modes of C-O bonds in glucose; when de-excitation occurs through nonradiative molecular transitions, the sample is locally heated, producing a temperature gradient and a material strain. The strain can be detected by an acoustic sensor. Localised heating and expansion of the material from a pulse of light produces a pulse of an acoustic wave.

[0006] The use of a photoacoustic detector for in vivo measuring blood glucose levels is disclosed in WO 91/18548. In this prior art, infrared light of two wavelengths in the MIR region is applied at two different locations to a person's skin. One wavelength is selected such that blood glucose shows a specific absorption and the other wavelength is selected such that there is no specific absorption by glucose. An acoustic detector detects the pressure difference between the locations where the different wavelengths are applied.

[0007] A simple arrangement for measuring blood glucose levels by infrared transmission through a person's finger is disclosed in US-5313941. This arrangement uses a filament infrared source and silicon photodetectors with filters to select a certain wavelength band from the source.

[0008] None of the above techniques has yet led to a practically usable device for noninvasive detection of glucose. At infrared intensities which are practically usable without unduly heating or even burning a person's skin, all the known techniques are not sensitive and reliable enough or are too bulky for daily use.

[0009] It is an object of the invention to provide a simple and reliable apparatus and method for noninvasive detection of a substance in a sample.

[0010] This object is solved by the apparatus and the method set forth in the independent claims. The dependent claims are directed to preferred embodiments of the invention.

[0011] Substances of interest such as glucose have covalent bonds with fundamental resonance frequencies in the mid-infrared region of the light spectrum, i.e. at frequencies corresponding to infrared light wavelengths from 2.5 to 25 μm (wavenumbers of 4000 to 400 cm^{-1}). Hence, the mid-infrared region of the absorption spectrum of these substances contains relatively narrow absorption lines specific to each individual substance. This is an advantage over the use of the near infrared region at wavelengths from 0.76 to 2.5μm where infrared absorption by the substances of interest is due to harmonics of the oscillating molecular bonds and absorption bands are broader, overlap each other, have smaller and wider peaks and it is thus more difficult to attribute absorption to the substance to be detected.

[0012] What was previously believed a disadvantage of noninvasive detection of substances such as glucose in body fluids or tissue by mid infrared spectroscopy, namely the high parasitic absorption of mid infrared light by water is overcome in accordance with claim 1 by detecting absorption through the photoacoustic effect and by using laser light at a plurality of discrete wavelengths.

[0013] The use of the photoacoustic effect for detecting infrared light absorption has the advantage of enabling detection of the substance in a noninvasive technique from within a sample even if light absorption by the sample is too high to allow detecting the substance from transmitted or reflected light.

[0014] Irradiating the sample with laser light of at least two distinct and discrete wavelengths at a peak or valley in the absorption spectrum of the substance to be detected in the sample has two effects. Firstly, these are the wavelengths where the absorbance is less dependent on wavelength variations and on a possible shift in

the absorption lines due to unknown other components in the sample. Secondly, unnecessary heating of the sample by light of other wavelengths such as in conventional spectroscopy with infrared sources emitting a broad range of wavelengths is avoided. The admissible light intensity can therefore be concentrated on the discrete wavelengths which offer the most accurate results.

[0015] The features of claims 2 to 4 improve the accuracy of the detection. A measurement at the same location of the sample in accordance with claim 4 avoids errors from sample inhomogeneities.

[0016] A preferred laser device includes a semiconductor laser having a quantum well structure as set forth in claim 5. Quantum well structures are made by alternating layers of different semiconductor material and form energy sub-bands wherein sub-band transitions are used for operation of the laser. The transition energy depends on the semiconductor material and on the layer thickness and can be adjusted to meet the wavelength requirements of the invention. One such laser device is the quantum cascade laser mentioned in claim 6. A description of the quantum cascade laser can be found in J.Faist: "Quantum Cascade Laser" Science, 264 (1994) 553 to 556.

[0017] Claim 7 relates to the measurement of the concentration of the detected substance.

[0018] A preferred embodiment of the invention will now be described with reference to the drawings, wherein

Figure 1 shows an apparatus for detecting and measuring glucose in vivo in a person's body tissue or blood,

Figure 2 shows absorption spectra of aqueous glucose solutions of different concentrations, and an absorption spectrum of distilled water,

Figure 3 shows the glucose solution spectra of Figure 2 with the spectrum of distilled water subtracted,

Figure 4 shows absorption spectra of milk with glucose and with fat in various concentrations, and an absorption spectrum of distilled water, and

Figure 5 shows absorption spectra of milk with glucose and fat in different concentrations with the absorption spectrum of milk without glucose subtracted.

[0019] The apparatus shown in Fig. 1 is suitable for detecting and measuring in vivo the glucose concentration in a person's body tissue 1 or blood. It includes a cavity 3 which is placed on a person's skin 2. Attached to the cavity 3 is a laser device 4 including a quantum cascade laser 5. Also attached to the cavity 3 is a piezoelectric transducer 6 acting as a microphone.

[0020] The quantum cascade laser 5 and the piezoelectric transducer 6 are connected to a control unit 7 comprising a microcontroller 8 and a display 9.

[0021] To detect and measure the glucose concentration in the body tissue 1, the microcontroller 8 in the control unit 7 drives the quantum cascade laser 5 so as to emit pulses of a laser beam 10 which penetrate the skin 2 and enter the body tissue 1. Where the laser beam 10 is absorbed in the body tissue 1, the tissue is locally heated. The thermal expansion resulting from the localised heating initiates an acoustic pulse and the pulsed laser beam 10 thus leads to a pulse train of acoustic signals 11 which originates in the region where the laser beam 10 is absorbed. The pulse train 11 propagates into the cavity 3 and is detected by the piezoelectric transducer 6. Preferably, the pulse frequency of the laser beam 10 is selected by the controller 8 so as to meet the acoustic resonance frequency of the cavity 3 which thus amplifies the acoustic pulses 11.

[0022] The microcontroller 8 determines the peak-to-peak amplitude of each acoustic pulse 11 detected by the piezoelectric transducer 6. The peak-to-peak amplitude is a measure of the absorbed energy of the laser beam pulse in the body tissue 1. Preferably, the microcontroller 8 discards a portion of each acoustic pulse which, in accordance with the travelling time of the pulse, originates from a portion of the body tissue 1 where no useful information on the glucose concentration is expected. For example, to disregard acoustic signals originating from absorption of the laser beam in the outer layers of the skin 2, the first part of each acoustic pulse is discarded and the peak-to-peak amplitude is obtained from later portions of the pulse.

[0023] The wavelength of the MIR laser light beam 10 is one where the absorbed energy depends on the glucose concentration in the body tissue 1. Moreover, the voltages and currents applied to the quantum cascade laser 5 are changed after a predetermined number of laser beam pulses so as to tune the laser 5 to a different wavelength where the absorbance depends again on the glucose concentration. In this manner, at least three different wavelengths in the mid-infrared range are sequentially scanned. Preferably, the selected wavelengths are at peaks and valleys, i.e. at relative maxima and minima of the absorption spectrum of glucose in body tissue, blood or water.

[0024] Fig. 2 shows absorption spectra of aqueous glucose solutions with 100, 200 and 300 mg glucose per dℓ water (i.e. per 0.1 ℓ water). Also shown is the absorption spectrum of distilled water (0 mg/dℓ glucose). Fig. 3 shows each spectrum of the glucose concentrations with the spectrum of distilled water subtracted. Thus, the spectra shown in Fig. 3 are those of glucose alone in a water environment. As can be seen from Fig. 3, absorption maxima occur at wavenumbers of 1151, 1105, 1080, 1036 and 992 cm^{-1} for example. And absorption minima occur at wavenumbers of 1181, 1140, 1094, 1066 and 1014 cm^{-1}. Preferably, the quantum cascade laser 5 is tuned to scan through all these wavenumbers one after the other with a number of

pulses for each wavenumber. If the laser used cannot be tuned over this range, the laser device 4 of this embodiment could be modified to include a plurality of lasers each for a specific wavelength or wavelength range, preferably on the same monolithic device.

[0025] The microcontroller 8 calculates the glucose concentration by a least square calculation referring to reference spectra such as shown in Fig. 2 or 3 for known glucose concentrations. The calculated concentration is displayed on display 9. Alternatively, the glucose concentration could also be calculated from an average of concentrations obtained from the absorptions at each wavelength relative to a reference absorption for a reference glucose concentration determined beforehand.

[0026] Preferably, the microcontroller 8 calculates also the error of the least square calculation, i.e. the root of mean square error and makes a selection of only portions of each acoustic pulse and a selection of those acoustic pulses which originate from laser beam pulses of selected wavelengths so as to minimise the error. The selection can be chosen by trial and error among a number of pre-prepared selections until the error is smaller than a certain value. Thereby, the measurement of the glucose concentration focuses on an area within the tissue 1 where the measurement is most reliable, for example a blood vessel.

[0027] The measurement principle of the present embodiment has been tested in the measurement of glucose concentrations in milk. Milk has been used as a testing solution instead of blood because it is readily available and resembles blood in that a number of substances are present which could potentially disturb the measurement. These substances are e.g. lactose, proteins and fat.

[0028] The absorption spectra of milk with 100 mg glucose per dℓ but different amounts of fat, namely 1.5, 2.5 and 3.5 percent fat are shown in Fig. 4 together with the absorption spectrum of distilled water. Fig. 5 shows absorption spectra of solutions with different glucose concentrations in milk of different fat concentrations, after a spectrum of a solution with 0 mg glucose per dℓ and 1.5 percent fat has been subtracted from each of them. The spectra have been obtained with a spectrometer made by Bruker.

[0029] The glucose concentration in an unknown solution can be obtained from spectra of known glucose concentrations (such as Figs. 4 and 5) by measuring absorbance values and by a partial least square fit (PLS). The PLS fit is based on an algorithm by Carl-Friedrich Gauss: A standard curve based on spectra of known concentrations is calculated such that the sum of the squared differences between the measured values of the unknown solution and the corresponding values in the standard curve is minimised. The concentration is derived from the thus calculated standard curve.

[0030] The spectra of Figs. 4 and 5 include absorption bands useful for glucose concentration measurements in the wavenumber region from 1181 to 960 cm^{-1}. In a comparative experiment, absorbance values of a solution were measured and taken for the PLS fit at all those wavenumbers from 1181 to 960 cm^{-1} which have been used for recording the spectra of Fig. 4 or 5. Then, the root of mean square error of cross-validation (RMSECV) which indicates the standard deviation of the measured values from the standard curve and thus indicates the prediction error for the glucose concentration was 3.44 mg/dℓ. However, obtaining the entire spectrum of the measured solution in a broad wavelength region caused problems with measurement duration and sample heating.

[0031] To overcome these problems, the infrared absorbance is obtained in accordance with the present embodiment at certain distinct wavelengths only. When, other than in the comparative experiment, absorbance is measured only at maxima with wavenumbers of 1151, 1105, 1080, 1036 and 992 cm^{-1} and at a minimum at 1181 cm^{-1}, the error (RMSECV) is 38.1 mg/dℓ. When further minima are selected at 1140, 1094, 1066, 1014 and 960 cm^{-1} and the absorbance measured at these minima is used for the PLS fit together with the absorbance values measured at the maxima and minima mentioned before, the RMSECV value is only 5.28 mg/dℓ. And the measurement can be completed in a short time without unduly heating the sample. Hence, selecting a sufficient number of minima and maxima for the absorbance measurement can maintain the error at a tolerable value close to the error achievable by using the entire spectrum, but still avoids the disadvantages of using the entire spectrum.

[0032] Measuring the absorbance at discrete wavelengths at maxima or minima of absorption bands means in practice to measure the absorbance of an infrared light beam having a bandwidth significantly smaller than the width of the corresponding absorption or transmission band. Preferably, the bandwidth of the light beam should not exceed 2/3, 1/3 or better 1/10 or 1/20 the width of the band of the absorption spectrum where the minimum or maximum is measured.

[0033] Hence, the embodiment measures light absorption photo-acoustically with laser light at a plurality of discrete individual wavelengths where the largest photoacoustic effect on the glucose concentration is expected. The photoacoustic effect allows measurement of the light absorbance by glucose even where virtually no light escapes again from the body tissue under investigation. And the use of discrete wavelengths allows sufficient laser beam power concentrated to these wavelengths while avoiding unnecessary heating of the body tissue through irradiation with other less favourable wavelengths. The preferred device for emitting the mid-infrared radiation at selected wavelengths, with sufficient intensity but limited overall power so as to avoid overheating of the body tissue is a semiconductor laser having a quantum well structure. If a sufficient number of individual wavelengths is used, it may be

conceivable to measure infrared absorption with other detectors, e.g. an optical detector such as a photodiode, rather than with the photoaccustic detector.

[0034] These measures allow noninvasive testing and monitoring of glucose concentrations. Hence, diabetics can conveniently monitor their blood glucose concentrations themselves at short intervals.

Claims

1. An apparatus for detecting a substance in a sample (1,2), comprising:

   a laser device (4,5) for irradiating the sample with a light beam (10) which penetrates into the sample,
   an acoustic detector (3,6) for detecting acoustic signals (11) originating within the sample from absorption of the light beam, and
   an indication unit (7) coupled to the acoustic detector for indicating the presence of the substance from the detected acoustic signals,
   characterised in that the laser device generates infrared light of at least two discrete wavelengths in the mid-infrared region, each wavelength at a different peak or valley in the absorption spectrum of the substance in the sample, and that the indication unit indicates the presence of the substance based on acoustic signals detected for each of said discrete wavelengths.

2. An apparatus according to claim 1, wherein said discrete wavelengths include one at a peak and one at a valley in the absorption spectrum.

3. An apparatus according to claim 1 or 2, wherein said discrete wavelengths include at least three distinct wavelengths each at a peak or valley in the absorption spectrum.

4. An apparatus according to any of claims 1 to 3, wherein said laser device (4,5) emits said at least two discrete wavelength as individual laser beam pulses at different times to the same location of the sample (1,2).

5. An apparatus according to any of claims 1 to 4, wherein the laser device includes a semiconductor laser (5) preferably having a quantum well structure.

6. An apparatus according to claim 5, wherein the semiconductor laser is a quantum cascade laser.

7. An apparatus according to any of claims 1 to 6, wherein said indication unit (7) includes calculating means (8) for calculating the concentration of the substance from the amplitude of the acoustic signal at each of said discrete wavelengths by a least square method based on a reference spectrum.

8. A method for detecting a substance in a sample (1,2) comprising the following steps:

   irradiating the sample with a laser light beam (10) which penetrates into the sample,
   detecting acoustic signals (11) originating within the sample from absorption of the laser light beam, and
   indicating the presence of the substance from the detected acoustic signals,
   wherein said laser light beam is generated at at least two discrete wavelengths in the mid-infrared region, each wavelength at a different peak or valley in the absorption spectrum of the substance in the sample, and the presence of the substance is indicated based on the acoustic signal detected for each of said discrete wavelengths.

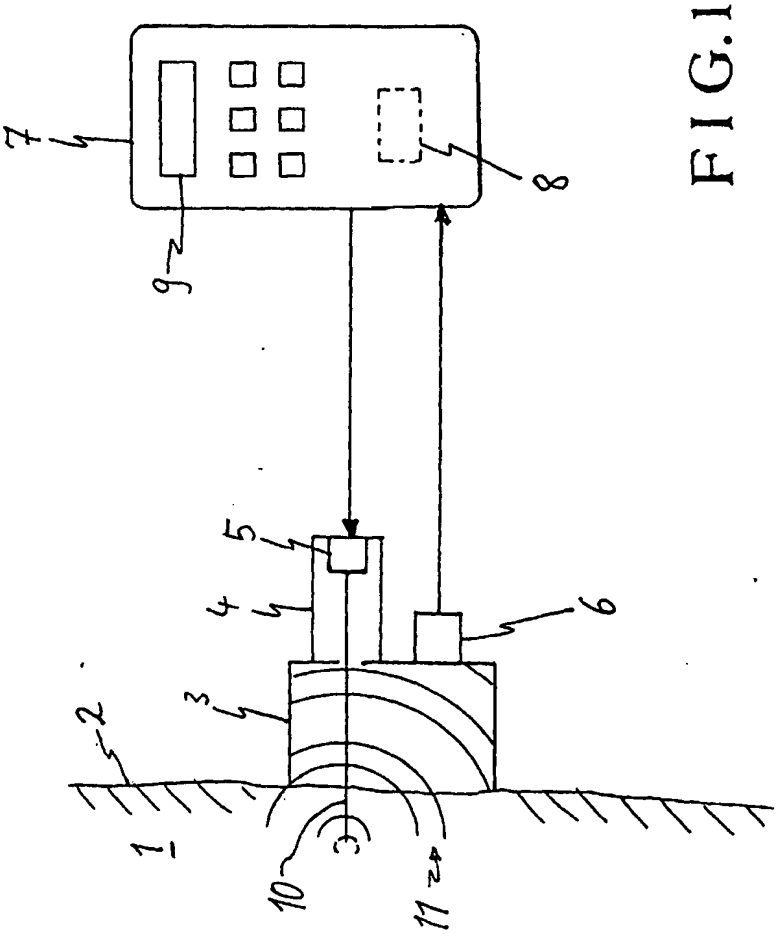

FIG.1

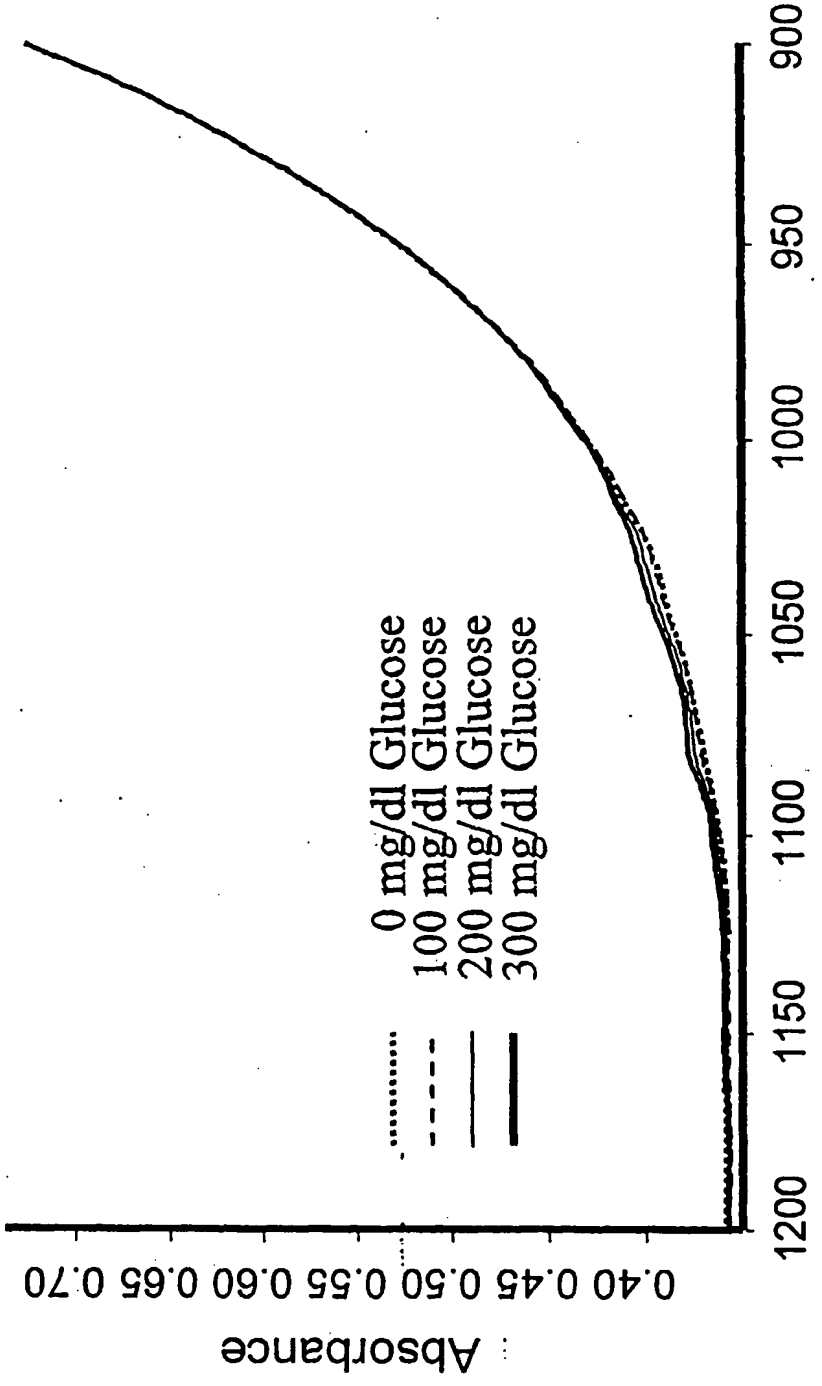
Absorbance
0.40 0.45 0.50 0.55 0.60 0.65 0.70
1200 1150 1100 1050 1000 950 900
Wavenumber [cm-1]
0 mg/dl Glucose
100 mg/dl Glucose
200 mg/dl Glucose
300 mg/dl Glucose
Glucose in H2O

FIG.2

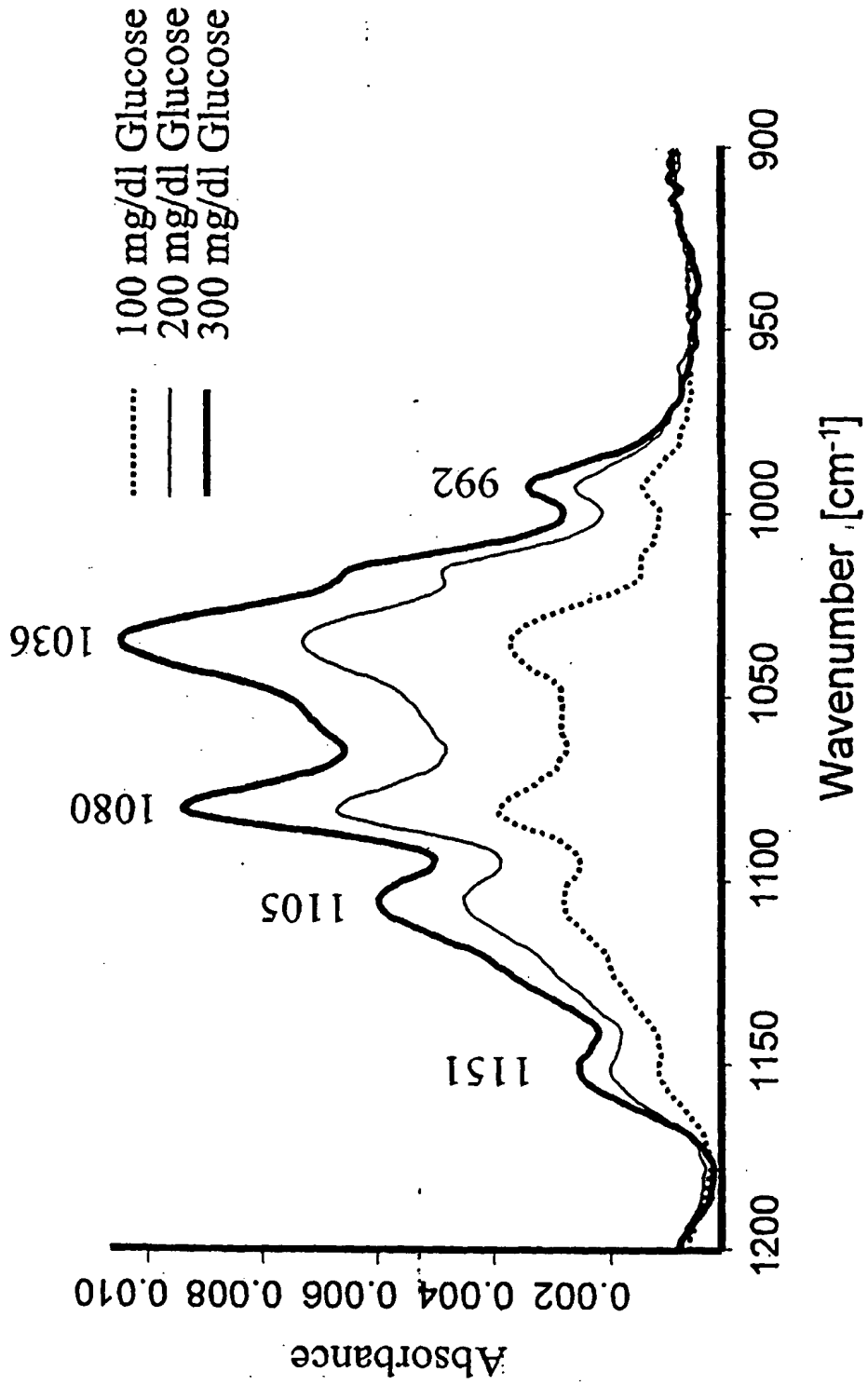
100 mg/dl Glucose
200 mg/dl Glucose
300 mg/dl Glucose
992
1036
1080
1105
1151
Absorbance
0.002
0.004
0.006
0.008
0.010
1200
1150
1100
1050
1000
950
900
Wavenumber [cm-1]

FIG.3

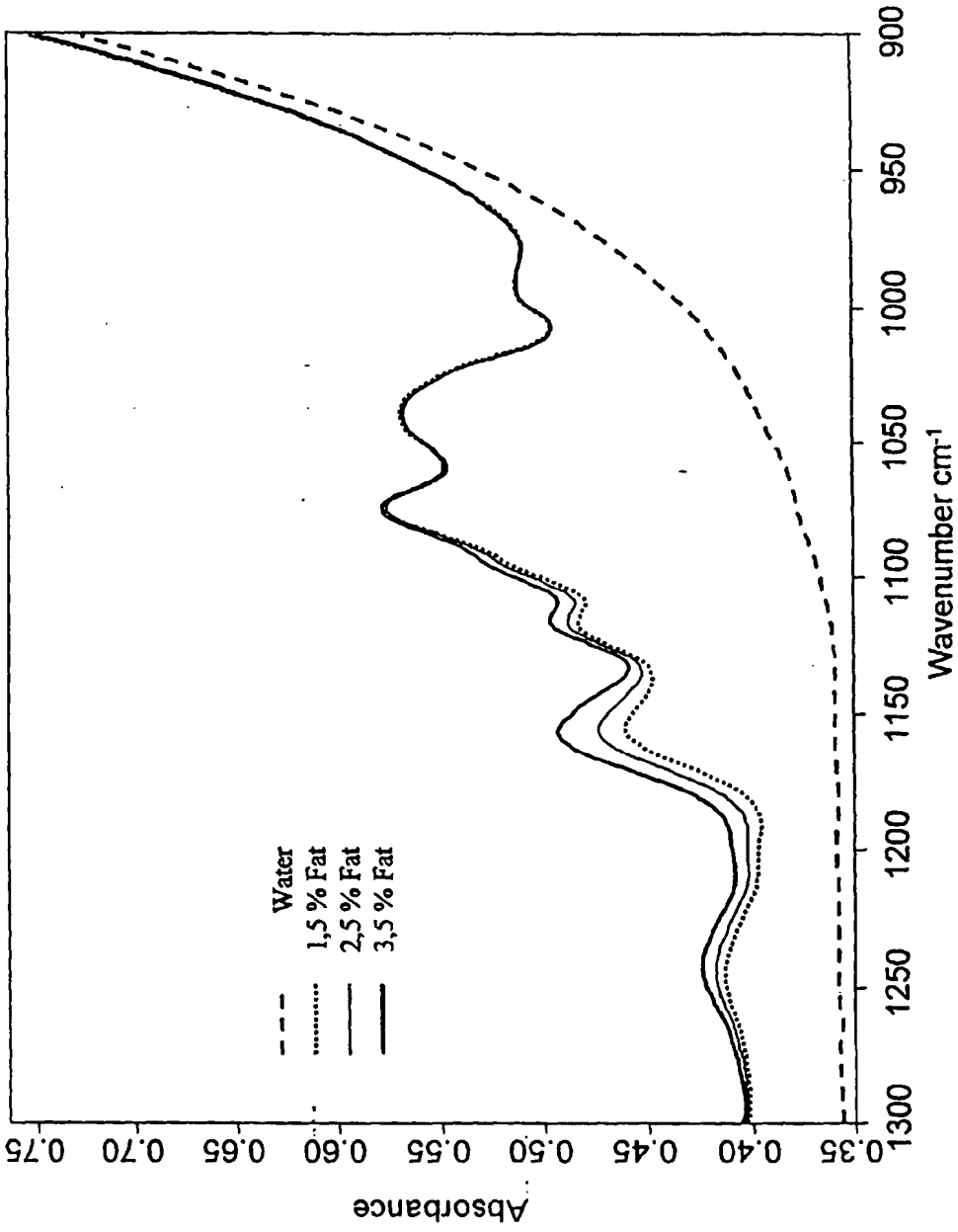
Absorbance
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
Wavenumber cm-1
900
950
1000
1050
1100
1150
1200
1250
1300
Water
1,5 % Fat
2,5 % Fat
3,5 % Fat

FIG.4

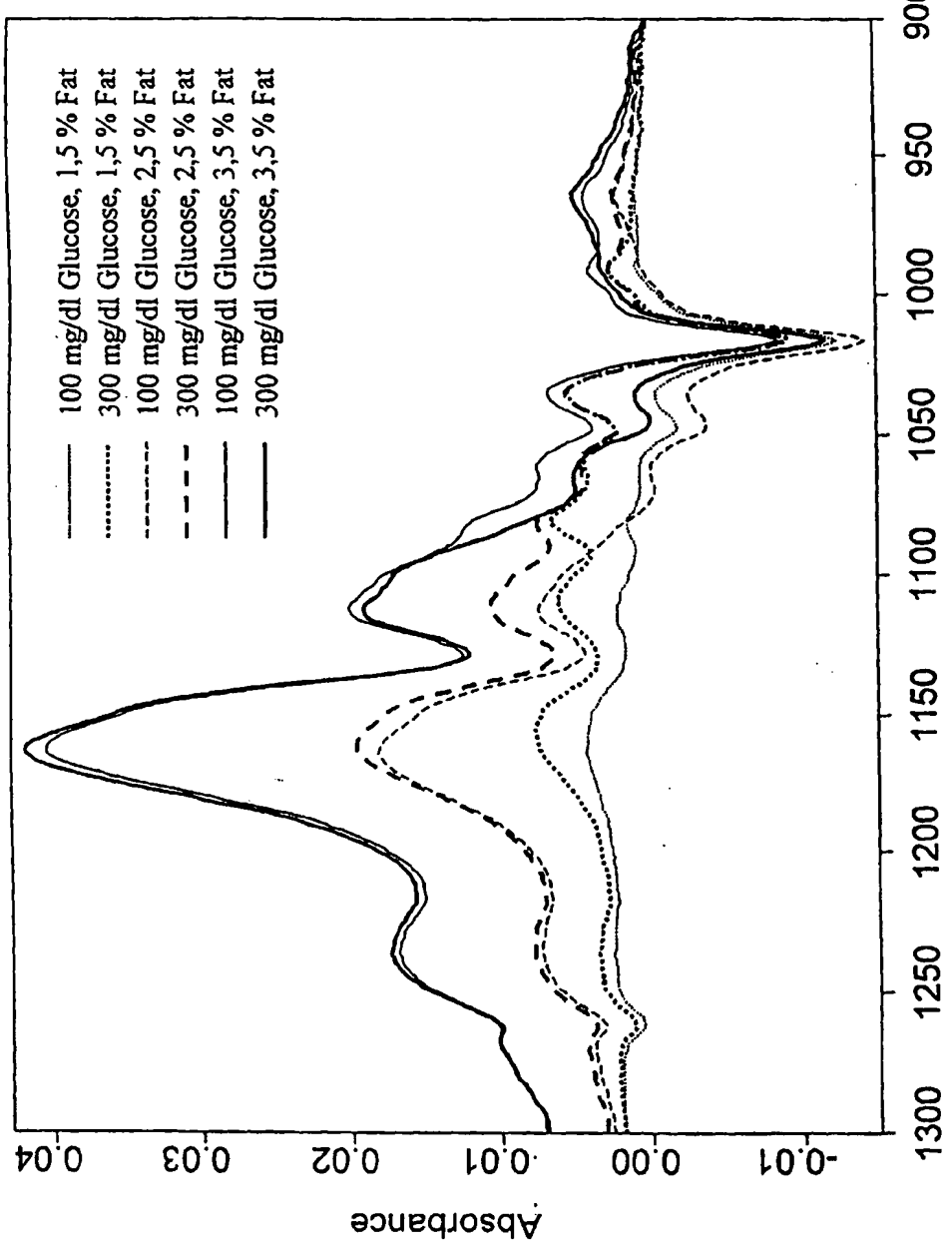
100 mg/dl Glucose, 1,5 % Fat
300 mg/dl Glucose, 1,5 % Fat
100 mg/dl Glucose, 2,5 % Fat
300 mg/dl Glucose, 2,5 % Fat
100 mg/dl Glucose, 3,5 % Fat
300 mg/dl Glucose, 3,5 % Fat
Absorbance
0.04
0.03
0.02
0.01
0.00
-0.01
1300
1250
1200
1150
1100
1050
1000
950
900
Wavenumber cm-1
Glucose-in-milk

FIG.5

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 00 10 8970

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
D,X	WO 91 18548 A (CLIFT VAUGHAN) 12 December 1991 (1991-12-12)	1,2,8	A61B5/00 G01N21/17
Y	* page 4, line 8 - line 20 *	1-5,8	
A	* page 12, line 12 - line 22 *	7	
Y	DE 44 46 390 C (SIEMENS AG) 4 July 1996 (1996-07-04)	1-5,8	
A	* column 3, line 27 - column 4, line 7 * * column 4, line 27 - line 37 * * column 6, line 46 - line 56 * * column 6, line 37 - line 39 *	7	
A	PALDUS B A ET AL: "PHOTOACOUSTIC SPECTROSCOPY USING QUANTUM-CASCADE LASERS" OPTICS LETTERS,US,OPTICAL SOCIETY OF AMERICA, WASHINGTON, vol. 24, no. 3, 1 February 1999 (1999-02-01), pages 178-180, XP000803131 ISSN: 0146-9592 * page 178, left-hand column, line 1 - right-hand column, line 3 *	1,4,5,8	

TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A61B
G01N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	15 August 2000	Knüpling, M

CATEGORY OF CITED DOCUMENTS

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15-08-2000

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82